THE PLEOMORPHIC STREPTOCOCCUS FROM THE AVERAGE THROAT AS A FILTERABLE VIRUS AND ITS RELATION AS SUCH TO EPIDEMIC POLIOMYELITIS.

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May 29,1919.

SUBMITTED TO THE DEPARTMENT OF BACTERIOLOGY AND THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF KANSAS IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS.

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Maude Leonard Howland.

It is now generally believed that the infection in epidemic poliomyelitis takes place through the medium of the naso-pharyngeal secretions. the virus has been shown to be present in the secretions (I) and in the tissues(2)&(3) of the naso-pharynx. Landsteiner and Levaditi(4) infected monkeys by inoculating the virus directly into the nasal mucosa.

Investigators believe that they have traced the virus to the olfactory lobe of the brain by way of the olfactory nerves(4),and others report instances in which the olfactory lobe was infected forty-eight hours after inoculating the virus intranasally, the medulla and spinal cord being unaffected. Healthy

individuals in contact with patients may have the virus in their naso-pharyngeal secretions(6) and Taylor andAmoss (7) report a case in which the naso-pharyngeal washings from a healthy child,who later developed paralysis, induced typical poliomyelitis in experimental animals. The same workers haveshown that washingsof the naso-pharynx inactivate or neutralize the active virus of poliomyelitis and that this power fluctuates in a given individual being especially diminished under inflamatory conditions of the upper respiratory tract(8).

Recent interest in epidemic poliomyelitis has centered around a pleomorphic streptococcus found commonly in the central nervous system, tonsils,and adenoids of poliomyelitic patients. Rosenow, and(9) othersin studies of elective localization of streptococcifound that the streptococci so constantly

present in poliomyelitis localizes in the central nervous system of young experimental animals and to a less degree in the central nervous system of adult animals, producing effects resembling those found in man. These investigators further found that cultivation on artificial media, especially under aerobic conditions, usually destroys promptly the elective localizing power. Furthermore, the streptococcus has been obtained as a filterable virus resembling the"globoid bodies" of Flexner and Noguchi.

Sherwood and Downs (II) have shown pleomorphic strains of streptococcus salivarius to be present in the nasopharynx of about 25% of normal persons. They have also shown that these organisms localize in the central nervous system of experimental animals producing loss of muscle tone, and paralysis. It now remained to investigate this organism from the normal throat as a filterable virus.

healthy individuals.

PURPOSE OF THE PRESENT STUDY.

The purpose of the present study was to take the streptococcus salivarius isolated from throats unexposed to any epidemic of poliomyelitis, grow it under anerobic conditions, and see if a filterable virus could be obtained which could be grown back under aerobic conditions to the larger form, and further to find what effect, if any, the inoculation of young rabbits would produce.

ORGANISM.

Inasmuch as the different investigators speak of the 'pleomorphic streptococcus' without giving anyfurther classification as a rule, and inasmuch as the different strains of streptococci are pleomorphic to a varying degree, it seemed advisable in these experiments to work with as nearly as possible a well defined group. The streptococcus most commonly andmarkedly pleomorphic seems to be the streptococcus sal-

ivarius o f Holman's classification, i.e. gram positive non-hemolytic, fermenting lactose but not mannite or salacin sugar media when inoculated from a twenty-four hour broth culture into which had been planted a colony from a twelve to twenty-four hour blood agar plate streaked with fresh material. This was the organism used in the following experiments.

PROCEDURE.

Nov. 21st. Blood agar plates were streaked with various throat swabs and on the following day small non-hemolytic colonies were picked into dextrose calcium carbonate broth.

Nov. 23rd. Lactose, mannite, and salicin serum agar tubes were inoculated from the broth cultures and other cultures were made for study. Sterile oil was then poured over the twenty-four hour broth cultures for

*See appendix.

anerobic growth.

Nov. 26th.The sugar media was inspected for fermentation and only cultures retained that had given the desired reactions,i.e.lactose positive, mannite and salicin negative.

The subcultures were examined daily until filtered and the original cultures were examined just before filtering. The material for microscopical examination was obtained by means of a capillary pipette thrust quickly through the layer of oil,the material being taken from near the bottom of the tube.

Of the cultures giving the desired reaction AA&AC were from the throats of persons subject to tonsilitis and who had recovered from attacks of influenza about two weeks previously.

MICROSCOPICAL STUDY.

TWENTY-FOUR HOUR AEROBIC GROWTH.— Smears from twenty-four hour aerobic growth showed numerous branching chain forms(PlateI),long and short chain forms, usually with longitudinal diplococcus grouping, lookinglike rods under light field but darkened field showed them to be closely adjusted diplococcus forms. There were chains composed of minute coccus forms and chains of larger coccus forms, the longer the chain thelarger the cocci as a rule, but considerable variation in size of the individual cocci in a chain, and few chains showed as many as IO-I5 cocci. A few pleomorphic diplococci were present and also a few staphlococcus groups were to be seen.

In the second group which was started anerobically December 1second serum was addedto dextrose meat-infusion broth. In these cultures there was a rapid initial growth, division taking place both transversely and longitudinally. Long chains two and three times the diameter of the

field were approximated in strands of from two tosix chains with the individual cocci and spaces lying parallel.

ANEROBIC GROWTH.

After fourty-eight hours the dextrose calcium carbonate broth cultures showed many fine diplococci and monococci,the cocci often being oval in outline. The individual cocci decreased in size until December 4th i.e.for eleven days anerobic growth,when the field became nearly blank.

The second group grown anerobically showed a speady breaking up of the long chains. In 72hrs. the chain formation was entirely destroyed. Monococci, diplococci, and a few staphlococcus groups were to be seen.

Sugar media was again inoculated to see if the same reactions would be given after anerobic growth

given by the initial aerobic growth. These were grown aerobically. Ac gave the same reactions as at first. AA after six days of anerobic growth varied in its reactions to sugar media for three or four days but returned to the characteristic reactions before filtering. During this timeAA was also hemolytic but was again non-hemolytic when it returned to the characteristic sugar reactions.*

INOCULATION.

Dec.4. AA&AC were filtered through a berkfeld filter and centrifuged. The supernatant fluid was pippett-

* This is significant in view of the fact that a swab from the throat of the same individual five weeks previously had given no hemolysis. Two weeks later this person had an attack of influenza and gave a pure culture of hemolytic streptococci in throat swab a week after apparent recovery. The strain used in the present study was taken a week later from the same throat and the swab gave no hemolytic streptococci.

ed off and discarded. Cultures were made in dextrose serum broth, ten per cent serum, two per cent blood agar and dextrose blood agar.

One cubic centimeter was inoculated intracerebrally a little to left of midline and into motor area of cerebral cortex of young rabbits AA&AC. The weight and temperatures of the rabbits were taken before inoculation and at intervals afterward. The results are given inthe following tables.

AA.

	Weight	Temp.	Remarks.
Dec.4th.	278gms.	99.0	
" 6"	272"	99.0	
" 7"	295"	97.0	
" II"	285"	96.0	Loss o f muscle tone in left hind limb.
" I4"	303"	96.2	Marked spreading of left hind limb.
" I6"	3I4"	I00.3	
" I8"	325"	99.0	Muscle tone improved. Less active. Muscular atrophy.
" 2IST.	292"	98.2	"" "

" 22nd.-----Died during the night.

Autopsy.-- Pleomorphic streptococci were found in the medulla and lumbar cord. Death was probably due to respiratory failure.

Results of inoculation of AC.

	Weight	Temperature.	Remarks
Dec. 4th.	342 gms.	97.6	Well and active when inoculated.
" 6th.	337 "	96.8	
" 7 "	330 "	100.00	
" 11 "	340 "	97.0	
" 14 "	394 "	96. 0	Marked loss of muscle tone.
" 16 "	417 "	97.2	
" 18 "	434 "	97.6	Muscle tone improved but muscle atrophy quite apparent altho otherwise gaining in weight.
" 21st.	462 "	97.4	
" 26th.	517 "	100 .6	

Muscle atrophy remained well marked although increase in weight was continuing daily.

Dec. 28th . AC was chloroformed and autopsied. Pleomorphic streptococci were found in the cerebellum but cultures became so contaminated that sugar reactions were were not obtained.

HVA acted like AC except there was complete recovery. The loss of muscle tone in this case was most marked on the third day.

MA had a loss of muscle tone and paralysis of left front legbegiming on the 5th day and lasting five days. During this time the limb was not used when the rabbit moved. A gradual and complete recovery followed. MA was inoculated intravenously. All others were intracerebrally.

With the exception of HVA there was a slight initial loss of weight after inoculation then a steady increase. AA lost weight the four days preceding death.

RECOVERY OF ORGANISM FROM FILTRATE.

AA was grown back to the larger form on dextrose blood agar. HVA was grown back on dextrose serum broth and MA was grown back on the same. AC gave a pure culture in a

portion of the filtrate enriched with a cc of serum. All incubations both aerobic in recovering the organism and anerobic before filtering were at 37.5°C.

SUMMARY.

The finding of pleomorphic streptococci commonly associated with epidemic poliomyelitis by numerous investigators led to the investigation of the streptococcus salivarius, the most constantly pleomorphic streptococcus, as a possible causative factor in this dread disease. The organism was found to be a filterable virus which could be grown back to the original size after being grown to a smaller form under anerobic conditions. The filtrate in one instance caused loss of muscle tone and was probably the cause of death of young experimental animal. In several instances it caused loss of muscle tone and in one case complete paralysis for a time. The organism was recovered from the different parts of the central nervous system.

CONCLUSIONS.

FIRST.* Streptococcus salivarius grown under anerobic conditions is a filterable virus.

SECOND.* The filterable virus of streptococcus salivarius has in several instances produced loss of muscle tone, in one case complete paralysis in experimental animal with recovery, and in one instance was the probable cause of death.

THIRD.* These experiments although not extensive give results inviting further investigation of the possible relation of streptococcus salivarius from the average throat to epidemic poliomyelitis.

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APPENDIX I.

The more important streptococci in Holman's Classification.

	Hemolysis	Lactose	Mannite	Salicin
S. pyogenes	±	±	−	±
S. anginosus	±	±	−	−
S. fecalis	−	±	±	±
S. mitis	−	±	−	±
S. SALIVARIUS	−	±	−	−
S. equinus	−	−	±	−

APPENDIX II.

MICROPHOTOGRAPHS OF STREPTOCOCCUS SALIVARIUS AT DIFFERENT STAGES OF THE EXPERIMENTS.

All microphoto graphs were taken under oil of immersion lens with total magnification of1200.

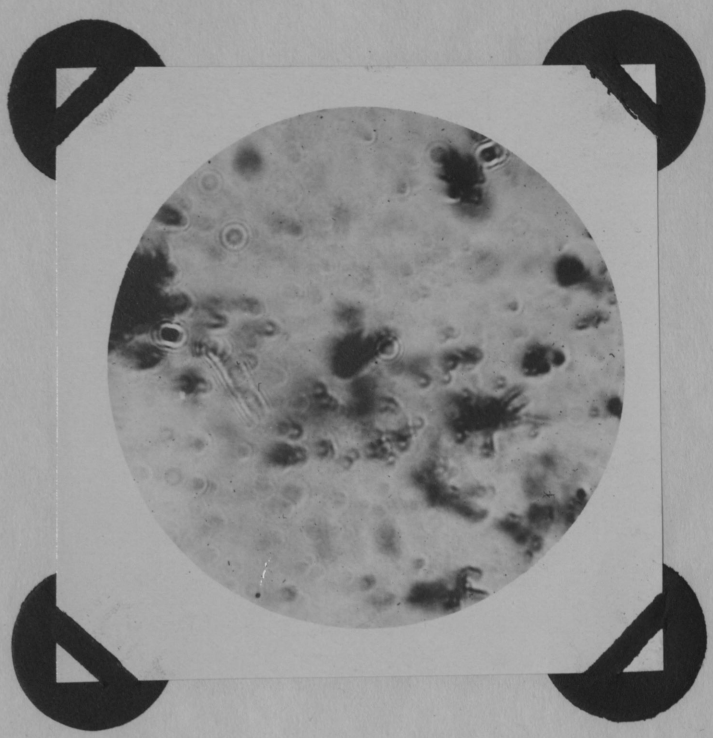

Fig. I. (Ix I200)

Fig.I. Morphology of throat smear from which AA was taken. In the center is a pod like structure which would seem to have some connection with method of multiplication. The different forms in which it appears under artificial cultivation ma makes it probable that other forms might exist in its human habitat. Only one short chain coming out of the "pod' shows in the microphotograph. Two other shorter chains in the base of the structure are distinct when viewed under the microscope.

Fig.2.(I200)

Fig.2&3. show branching chain forms. Fig.3. also shows variation in size of organisms.

Fig.3(IXI200).

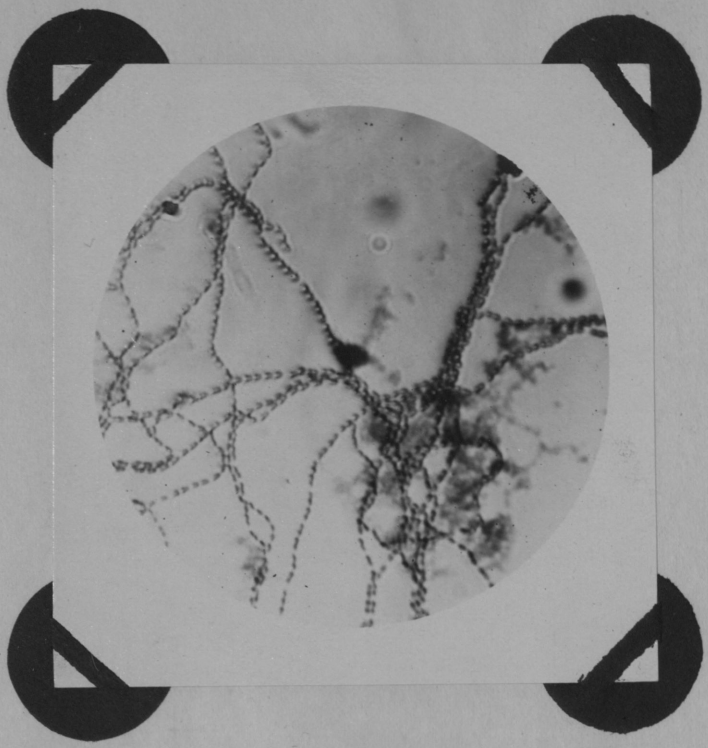

Fig.4.(1200)

Fig.4. Dextrose serum Ca CO_3 broth culture at 48hrs. Frequently three of these long chains were approximated, the middle one almost always smaller than the other two. Where two were approximated for only a few coccus forms the cocci in one chain were usually smaller than the other where approximated although being practically equal in the two chains throughout where not touching each other.

Fig.5. Occasionly a chain was found where division seemed to be taking place longitudinally throughout the entire length of a chain. This also was at 48hrs.

Fig.5.(1200).

Fig.6.(IXI200)

Fig. 6. AA two days before filtering. This is a pure culture of fine diplococci with a very few monococci. The organisms were so fine that they look like mere shadows in the microphotograph.

Fig.7. Just before filtering. Only a few monococci could be seen under oil of immersion lens.

These did not show up in the micro-photograph.

Fig.7.(IXI200).

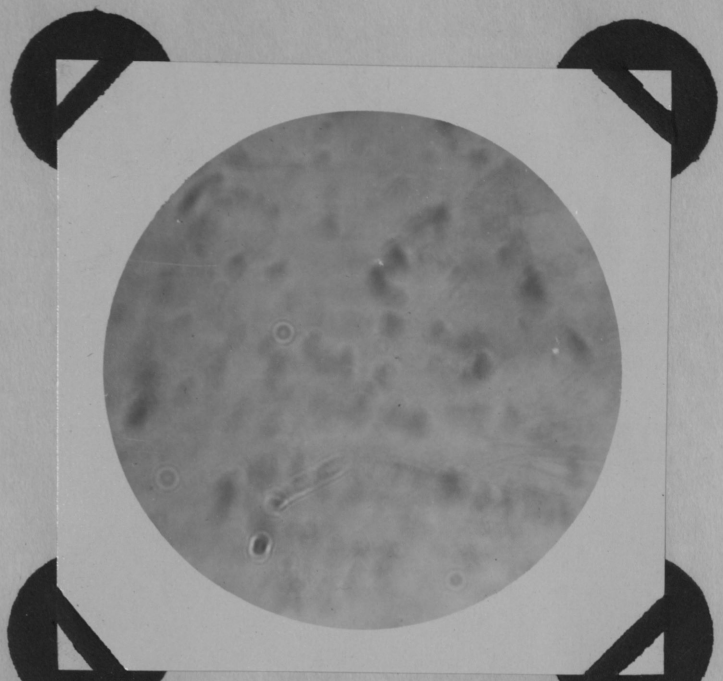

Fig.8.(IxI2oo)

Just after filtering.

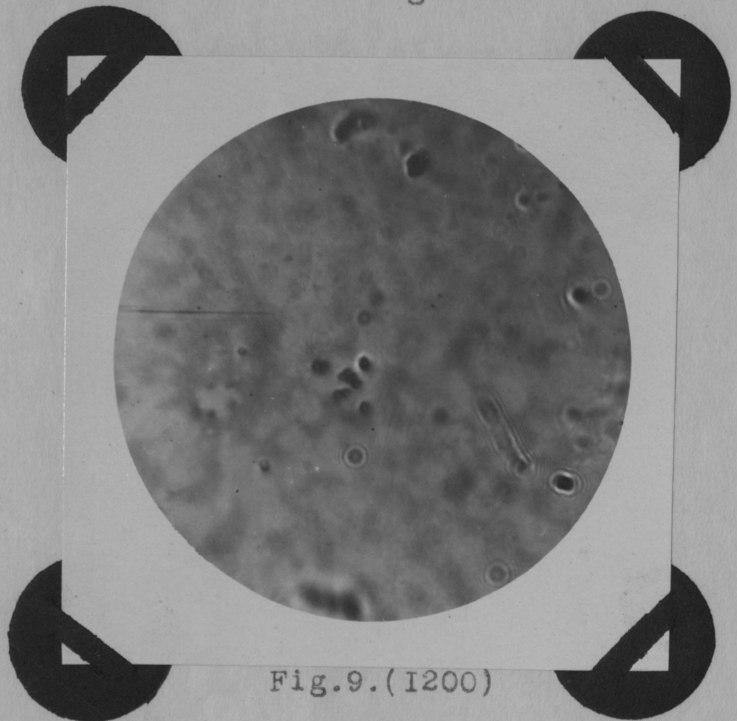

Fig.9.(I200)

24hr. dextrose $CaCO_3$ broth culture from above filtrate.

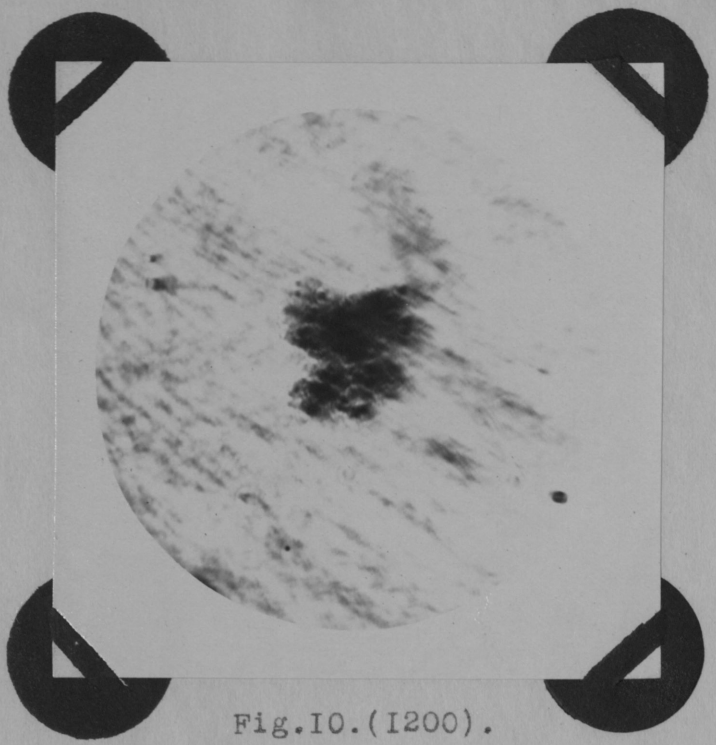

Fig.IO.(I200).

Organisms in cerebrum. The figure shows the organisms clumped together with what looks like broken up cells. The stain used on this slide was methylene blue so nn nerve cell do not show in microphotograph.

Fig. II.Organisms cultivated on Dex. blood agar from the medulla of